

# SUCROSE (TOTAL GLUCOSE) Liquid

UV DETERMINATION IN WINE, FOOD & BEVERAGES

Kit: 52 mL

Cod. SU9919

## PRINCIPLE

In the presence of ATP, NADP,  $\beta$ -Fructosidase, G6PDH (glucose-6P-dehydrogenase) and HK (hexokinase), Sucrose and glucose produce NADPH. The intensity of the UV-colour at this wavelength is proportional to the conc. of TOTAL GLUCOSE (Sucrose+Glucose) in the sample.

## REAGENTS

Components of the kit:

	<b>Cod. SU 9919</b>
<b>*REAGENT 1</b> (buffer, liquid, ready to use)	<b>1 x 40 mL</b>
<b>*REAGENT 2</b> (liquid)	<b>1 x 9 mL</b>
<b>*REAGENT 3</b> (suspension)	<b>1 x 3 mL</b>
Good buffer > 20 mmol/L	HK > 10 U/L
NADP > 0.2 mmol/L	G6PDH > 5 U/L
ATP > 2 mmol/L	$\beta$ -Fructosidasi > 1 KU/L
<b>*REAGENT 4</b> (liquid, ready to use)	<b>1 x 3 mL</b>
Standard 1 g/L	

STABILITY: the reagents, at 2-8°C, are stable up to the expiry date shown on the package **if not contaminated during handling.**

## PREPARATION OF THE WORKING REAGENT

Draw all the volume of \*Reagent 3 adding to the bottle of

\*Reagent 2. Mix kindly without foaming.

Let the reagents reach the working temperature before use.

**Close immediately after handling. Incompetent handling will release us from any responsibility.**

STABILITY on board of the instruments: 1 month at 2-8°C.

## SAMPLE

- Wine could be used directly.
- Use colourless, clear and quite neutral liquid samples directly if Sucrose conc. is between 0.030–1.000 g/L; otherwise, dilute with water to reduce it in this range.
- Turbid solutions have to be filtered or centrifuged.
- Samples containing carbon dioxide have to be degased.
- Acid samples have to be adjusted by adding KOH /NaOH until approx. pH 8 is reached.
- Alkaline samples have to be adjusted by adding HCl until approx. pH 8 is reached.
- Strongly coloured samples have to be treated with PVPP (polyvinylpyrrolidone e.g. 1 g/100 mL Sample).
- For other different samples, please inquire the use and for potential pre-treatment.

## PROCEDURE

• Wavelength:	340 nm (334-365 nm)
• Pathlength:	1 cm
• Reading:	against air or distilled water
• Temperature:	37°C
• Method:	end-point
• Reaction:	10 minutes
• Linearity:	0.030 – 1.000 g/L
• Sample/reagents:	1/40/10

**Let reagents reach the working temperature before using.**

Pipette in a test tube or cuvette so labelled:

R/B: Reagent Blank, St: Standard, S: Sample:

	R/B	St	S
*Reagent 1	1000 $\mu$ L	1000 $\mu$ L	1000 $\mu$ L
Distilled water	25 $\mu$ L	---	---
*Reagent 4 (St)	---	25 $\mu$ L	---
Sample	---	---	25 $\mu$ L

Mix kindly and incubate for 5 minutes at 37°C. Measure the absorbances of Sample (AS0) and Standard (Ast0) against the Reagent Blank (AR/B0). Then add:

*Working Reagent	250 $\mu$ L	250 $\mu$ L	250 $\mu$ L

Mix kindly and wait the end of the reaction (5 min).

Read respectively AS1, Ast1 and AR/B1.

Calculate for the Total Glucose (Sucrose+Glucose):

AS=(AS1 - AS0) Ast=(AS1 - AS0) AR/B=(AR/B1 - AR/B0)

**Calculate  $\Delta AS = AS - AR/B$  and  $\Delta AST = Ast - AR/B$ .**

## CALCULATION for TOTAL GLUCOSE (SUCROSE+GLUCOSE)

Use this general formula to calculate the concentration:

**$[\Delta AS / \Delta AST] \times \text{Standard conc.} = \text{TOTAL GLUCOSE (g/L)}$**

## CALCULATION for SUCROSE

This proposed method determines together Sucrose + Glucose, eventually available in the sample.

The determination of Sucrose free from Glucose in the same sample, might be done subtracting the Glucose determined by a different Reagent (our Cod. GL9916), from the Total Glucose determined by this present Reagent; considering of course the different Molecular Weight (MW) of the two sugars.

For instance:

**SUCROSE = TOT. GLUCOSE - GLUCOSE x 1.90**

where 1.90 = MW Sucrose / MW Glucose = 342.3/180.2

So if we had

Total Glucose = 1.600 g/L and Glucose = 0.500 g/L

**Sucrose = 1.600 - 0.500 x 1.90 = 0.650 g/L**

## NOTE

1. A proportional variation of the reaction volumes does not change the results.
2. We suggest do not mix Reagents from different Production lots.
3. For concentrations higher than 1.000 g/L dilute the sample with distilled water in the range 0.030-1.000 g/L, repeat the determination and multiply the result by the dilution factor.
4. **PAY ATTENTION!** Applications on routine Analyzers may be totally different from what we developed as manual determination, and also from themselves.
5. For fat containing samples please ask for specific procedure.
6. For solid or semi-solid samples please ask for specific procedure, eventual Carrez solutions pretreatment and Calculation.

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